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RESEARCH ARTICLE

Niche differentiation between *Malus sylvestris* and its hybrid with *Malus domestica* indicated by plant community, soil and light

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Abstract

Question: *Malus sylvestris* is considered an endangered tree species in Central Europe. Hybridization with *Malus domestica* poses a serious threat to the genetic integrity of the wild species. Here we investigate whether *M. sylvestris* and the hybrid *M. domestica* × *sylvestris* occur in the same habitat or have different ecological niches and whether *M. sylvestris* is threatened by displacement by the hybrid.

Location: Northern Bavaria.

Methods: Taxon delimitation was accomplished using six genetic microsatellite markers and 613 Germany-wide references of *M. sylvestris* and 75 cultivars. To determine differences in the ecological niches between *M. sylvestris* and hybrids, light availability for the trees was estimated via gap fractions in hemispherical photographs. Soil particle size fractions and pH values were determined for each horizon. Vegetation relevé data were collected, and mean Ellenberg indicator values calculated. For habitat differences, means in combination with frequency patterns of the parameters were compared, and logistic models and detrended correspondence analysis (DCA) of community data were calculated.

Results: Genetic markers identified 22 *M. sylvestris* and 11 hybrid specimens, meaning that in the study area the wild taxon is much more frequent than the hybrid. Ecological site differences between *M. sylvestris* and its hybrid with *M. domestica* were best explained by light availability, pH and mean Ellenberg moisture value. In contrast to the ecological demands of the hybrid, *Malus sylvestris* tolerated wet soil and flooding and even somewhat shadier conditions in the later successional stages. DCA revealed that differences in the composition of the plant communities in which the taxa were found were primarily driven by soil moisture.

Conclusions: Our data suggested different ecological niches, which are appropriate to reduce the risk of replacement of *M. sylvestris* by the hybrid *M. domestica* × *sylvestris*. Hence, these findings provide important implications for a more targeted planning of in-situ conservation strategies of *M. sylvestris* genomes with low levels of admixture and help to protect plant communities suitable for the threatened wild apple.

KEYWORDS
community exclusion, ecological niche, European wild apple, genetic characterization, hybridization, *Malus domestica*, *Malus sylvestris*, replacement by hybrids, understory tree

1 | INTRODUCTION

Malus sylvestris (L.) Mill., the European wild apple, is a rare tree species distributed from Europe to Asia minor and the Caucasus (Wagner, 2005). With a height of 8–15 m it is a comparably small tree species belonging to the second or third tree layer (Stephan et al., 2003; Wagner, 2005; Aas, 2013). In Germany the wild apple is very rare and threatened for different reasons. One important threat is hybridization with the domesticated apple (Spethmann, 1997; Allendorf et al., 2001). *Malus domestica* Borkh. is cultivated everywhere and often adjacent to the habitats of the wild apple. Apple species appear to have the poor genetic isolation mechanisms typical of many Rosaceae species (Wagner et al., 2014). It can easily hybridize with the domesticated apple due to the lack of prezygotic isolation mechanisms (Larsen et al., 2008). Seed set of *M. sylvestris* could be even higher through cross-pollination with *M. domestica* than via intraspecific pollination in cases where the gene pool of small *M. sylvestris* populations is strongly limited because of the presence of so-called self-sterility factors (Larsen et al., 2008, Höltken et al., 2014). Morphologically, it is difficult to discriminate accurately between *M. sylvestris* and its hybrid with *M. domestica*, *M. domestica* × *sylvestris*. Therefore, genetic microsatellite data are often used to get more exact results, as done in this study to verify the morphological identification.

The European wild apple is adapted to diverse ecological habitats (Aas, 2013). For example, the versatile and abiotic stress-resistant wild apple is able to survive in floodplain forests, as well as on calcareous limestone outcrops (Walentowski et al., 2018). It occurs also on the edge of forests or in cultivated habitats like thickets or in hedges (Leuschner & Ellenberg, 2017). *Malus domestica*, the domesticated apple, was brought from Asia to Europe via the Silk Route about 4,000 years ago. *Malus domestica* is derived from the *M. sieversii* (Ledeb.) Koidz., which is distributed in Central Asia (Janick et al., 1996; Velasco et al., 2010; Cornille et al., 2012). A contribution of *M. baccata* Loisel., *M. orientalis* Uglitzk, ex Juz. and also *M. sylvestris* has also been proven (Wagner & Weeden, 2000; Robinson et al., 2001; Harris et al., 2002; Harrison & Harrison, 2011; Cornille et al., 2012). The proportion of *M. sylvestris* genes differs greatly depending on the cultivar of the domesticated apple, however (cf. Cornille et al., 2012). It is known from other taxa that habitats of hybrids are often different from those of either parental species (Cruzan & Arnold, 1993; Rieseberg et al., 2003) and it is likely that the niches of *M. domestica*, *M. sylvestris* and the hybrid between these two species are different. Characteristics of *M. domestica* were influenced mainly by breeding for its use as a crop, which is focused on the development of resistant trees that can be grown in orchards in full overhead light and under the absence of a tree cover. Moreover, the focus is on fruit yield and quality. In contrast, the hybrid between the domesticated and the wild apple occurs spontaneously, in contrast to the widespread planting of *M. domestica* trees. Furthermore, the hybrid — as is the wild apple itself — is exposed to natural selection and competitive exclusion. Natural selection and breeding selection may lead to different ecological optima and amplitudes.

The question arises whether the rare wild apple could be replaced by its hybrid with the domesticated apple which in Central Europe is planted nearly everywhere in close proximity to the wild species (Wolf et al., 2001). Detailed synecological research on apple cultivars or their hybrids with the wild apple has not been carried out yet.

According to Kay et al. (2018) niche differentiation between coexisting relatives can take the shape of both pre- and post-zygotic reproductive barriers. In our study, the post-zygotic barriers, such as selection against migrants between habitats and extrinsic selection against hybrids, are of particular interest. In our case study area, the hybrid is still comparatively rare and *M. sylvestris* dominates in numbers and frequency. From our field work, we have the impression that the reason for the rather low number of hybrids could be that the habitats of *M. sylvestris* are not equally suitable for the hybrid's survival. If hybrids are ecologically excluded from the habitats of the wild apple it might be possible that despite a high cross-species seed set a low rate of hybrid establishment occurs and post-zygotic barriers become effective (Kay et al., 2018). This in turn could reduce the risk of total replacement of *M. sylvestris* by *M. domestica* × *sylvestris*.

By this ecological exclusion, the frequency of *M. sylvestris* could be increased locally. To investigate the role of ecological exclusion we compared the niches of both *M. sylvestris* and the spontaneously occurring *M. domestica* × *sylvestris*. Sites of both taxa were compared via selected abiotic and biotic factors which may contribute to niche differentiation. The study area in northern Bavaria is relatively small and is characterized by a variety of different habitats of *M. sylvestris* and its hybrid, thus providing the basis for an efficient study of ecological exclusion (see Kraft et al., 2015); among them are the typical habitats of *Malus sylvestris* such as flood plains and early- and mid-successional forest stages. Analyses of abiotic factors concentrated on soil (physico-chemical) parameters and light availability. We measured gaps in the canopy of the upper tree layer to test light differences which might constrain the occurrence of the apples, since they are usually limited to the understorey and to the shrub layer. Biotic factors were assessed by analysis of plant community data. Ellenberg indicator values (EIVs) reflect the current local site conditions resulting from long-term effects very properly and they have a long tradition in the interpretation and understanding of plant communities and their evolution (Schaffers & Sýkora, 2000). Soil and light differences were analysed by pairwise comparisons of means and medians. These values and EIV data were used to build logistic models.

Here we aim to answer the question whether there are detectable differences in site ecological characteristics between *M. sylvestris* and its hybrid with *M. domestica* that may constrain and exclude the hybrid from core habitats of *M. sylvestris*, thus preventing the extinction of the latter.

2 | METHODS

2.1 | Study sites

Three mixed sites with 22 *M. sylvestris* and 11 *M. domestica* × *sylvestris* specimens within a 15-km radius around the city of Bayreuth in Upper Franconia (northern Bavaria, Germany) (Figure 1) were studied from late spring to late summer in 2017. The sites were named BB, DS and RMA (Table 1). The localities represent different habitats on different geology, ranging from stone-rich marl and limestone bedrock over sandy riparian forests to clayey argillite.

There is a bias in the number of *M. sylvestris* and the hybrid per locality. Whereas it was equal for BB, in DS and RMA the number of *M. sylvestris* exceeded that of the hybrid. To avoid statistical bias from locality effects, we tested for overall locality differences in the investigated parameters (see below).

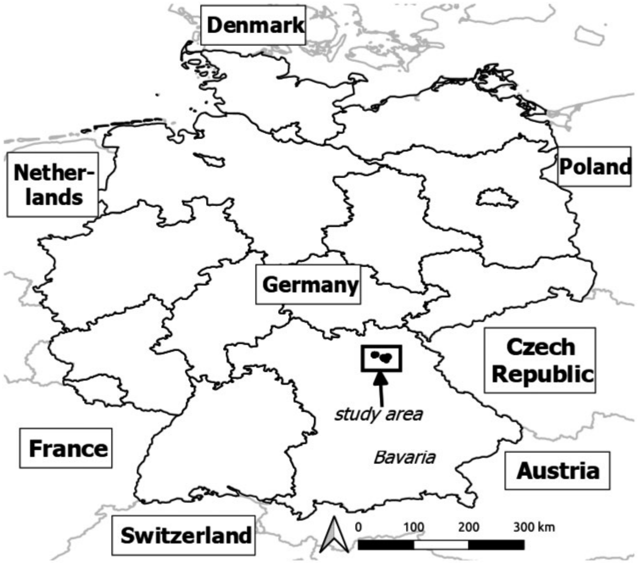


FIGURE 1 Location of sampling sites

2.2 | Identification of *Malus sylvestris* and hybrids

The delimitation between *M. sylvestris* and its hybrid with *M. domestica* is not trivial. Here, we used morphological features such as the pubescence of the leaf underside including the leaf's veins and costa for preliminary identification in the field. *M. sylvestris* has a glabrous leaf underside, whereas the hybrid has slightly to densely hairy leaves below (Reim et al., 2012; Wagner et al., 2014).

For genetic identification, we analysed six nuclear microsatellites (Appendix S1). Afterwards, we used STRUCTURE 2.3.4 software (Falush et al., 2003, 2007; Pritchard et al., 2009) which allows grouping of individuals into different taxonomic units (species or subspecies, hybrid proportions). The reference data set comprised 618 potential wild apple trees from a Germany-wide joint research project as well as the 66 most important cultivated apple varieties (for more information on the reference material see Höltken et al., 2014). A threshold of 5% was used in the STUCTURE analyses for group affiliation (either *M. sylvestris* or hybrid).

The genetic admixture proportions in STRUCTURE were estimated under the assumptions of existing genetic exchange ("admixture model") and correlated allele frequencies between the populations ("correlated allele frequency model"). Ten runs each were performed to estimate admixture proportions, assuming one to six clusters each ($K = 1–6$). Each run consisted of Monte Carlo Markov chains with 10,000 generations "burn-in" and 10,000 sampled generations. Based on this information, the conditional probabilities for placing individuals into a given number of clusters (here $K = 1–10$) were calculated (Evanno et al., 2005). The on-line version of STRUCTURE Harvester 0.6.5 (Earl and von Holdt 2012) was used for this purpose.

2.3 | Vegetation inventory

The size of the plots was fixed at a square of 100 m², with a *Malus* tree as the centre point of each plot. Cover values of vascular plants and bryophytes refer to the vertical projection of the above-ground living parts only (aerial cover) overhanging the surface of the sampling unit. The following layers were distinguished: (a) the tree layer from about 5 m upwards, comprising the top stratum, and consisting of phanerophytes. This layer was divided into the first (topmost), the second (middle) and the third tree layer (lowest). (b) The shrub layer with heights of about 1.5–5 m, consisting of young trees and shrubs, was divided into the first (upper) and second shrub layers (low bushes). (c) The herb layer, reaching heights of up to about 1.5 m. The herb layer consisted of various herbaceous plants, dwarf shrubs as well as young shrubs or tree saplings; (d) The moss layer (ml) growing on the surface of the forest floor.

Occurrences of species in discrete special habitats like epixylic bryophytes were excluded. To minimize biases of subjective judgement and errors related to that we ensured that all relevés in both areas were recorded by the same person. However, this also meant

TABLE 1 Coordinates of the sites and trees

			Coordinates (Gauß–Krüger)	
Site code	Taxon	Releveé names	GPS north	GPS east
BB	*M. sylv.*	B1	49.95734	11.42226
BB	*M. dom.* × *sylv.*	B2	49.95642	11.42183
BB	*M. sylv.*	B3	49.95643	11.42177
BB	*M. sylv.*	B8	49.95128	11.41884
BB	*M. sylv.*	B9	49.95087	11.41878
BB	*M. dom.* × *sylv.*	B10	49.95642	11.42181
BB	*M. sylv.*	B11	49.95725	11.45455
BB	*M. sylv.*	B12	49.94957	11.41832
DS	*M. sylv.*	D17	49.9048	11.56772
DS	*M. dom.* × *sylv.*	D4	49.90584	11.56766
DS	*M. sylv.*	D5	49.9064	11.56957
DS	*M. sylv.*	D6	49.90549	11.5691
DS	*M. sylv.*	D8	49.90501	11.56895
RMA	*M. sylv.*	R1	49.87881	11.61715
RMA	*M. sylv.*	R2	49.88386	11.62056
RMA	*M. sylv.*	R3	49.88388	11.6211
RMA	*M. sylv.*	R4	49.9214	11.63415
RMA	*M. sylv.*	R5	49.88425	11.61396
RMA	*M. sylv.*	R6	49.90642	11.61933
BB	*M. dom.* × *sylv.*	HB1	49.95627	11.42178
BB	*M. dom.* × *sylv.*	HB2	49.95117	11.42117
BB	*M. dom.* × *sylv.*	HB4	49.95201	11.42053
BB	*M. dom.* × *sylv.*	HB5	49.95265	11.41556
DS	*M. dom.* × *sylv.*	HD1	49.90133	11.58947
DS	*M. sylv.*	HD2	49.89956	11.57478
DS	*M. sylv.*	HD3	49.90572	11.5617
DS	*M. dom.* × *sylv.*	HD4	49.90553	11.56953
DS	*M. sylv.*	HD5	49.90637	11.56962
DS	*M. sylv.*	HD6	49.90488	11.56781
DS	*M. sylv.*	HD7	49.90488	11.56782
RMA	*M. dom.* × *sylv.*	HR1	49.90881	11.61911
RMA	*M. sylv.*	HR3	49.92061	11.65021
RMA	*M. dom.* × *sylv.*	HR4	49.88424	11.61392

RMA (Rotmainaue), DS (Destuben) and BB (Busbach) indicate the three studied populations around Bayreuth. The abbreviations *M. sylv.* stands for *Malus sylvestris*, *M. dom.* × *sylv.* for the hybrid with *M. domestica*.

that the data collection lasted several months and later records may suffer from low recognizability of spring forest geophytes.

Data such as sampling location, recording date, Gauß–Krüger coordinates, altitude above sea level, aspect, slope and percent cover of vegetation layers were documented in the sampling protocol. GPS coordinates were marked with a Garmin eTrex 30× (Garmin Ltd., Schaffhausen, Switzerland) and hill exposition was determined via the compass function of the GPS device, hill slope via the inclination function of a hypsometer. Elevation above sea level was transcribed.

2.4 | Gap fractions

Malus sylvestris is usually found in the second or third tree layer (understorey) below the emergent layer. Therefore, the availability of overhead light has to be interpreted as a combination of biotic and abiotic factors. As a stand-in for light availability, gap fraction of the stand was measured. Gap fraction is here defined as direct visibility of the sky from below the canopy at the edge of the *Malus* tree's crown. Gap fraction was measured via hemispheric photographs using a Nikon Coolpix 995 (CCD optical sensor) with a Nikon Fisheye

Converter FC-E8 0.21x (both by Nikon Corporation, Chiyoda, Tokyo, Japan) mounted on a tripod about 90 cm above the ground. Exposure time and relative aperture were set to fit the conditions in order to yield optimal results. The photographs were taken at the edge of the crown in each main cardinal direction facing directly upwards, resulting in four pictures per plot.

2.5 | Soil samples

Horizon-wise soil samples were taken from Pürckhauer soil auger drill cores. In-the-field analysis on these cores included determination of soil type, percentages of soil skeleton, and of fine soil (sand, silt and clay fractions in percent) using the finger method (standard methods of Sponagel et al., 2005). At every tree site, two soil cores were taken in each plot, if possible, one uphill and one downhill drilling core at a distance of about 1 m from the trunk. Additionally, soil samples were stored in polyethylene bags and analysed in a laboratory of the Bayreuth University. The soil samples were air-dried and soil aggregates were broken in a mortar. Samples of 2 g of stone-free soil were suspended in 8 ml distilled water. The samples were swayed for 19–20 h in an automatic horizontal swayer (SM 30, Edmund Bühler GmbH, Hechingen, Germany) at 100 motions per minute. The sediment was shaken by hand and then allowed to settle again for half an hour. pH was measured in the supernatant suspension with a Mettler Toledo InLab Expert DIN pH electrode (Columbus, OH, USA). Soil-chemical research was focused on soil pH, measured in the organic layer, topsoil and subsoil. They were selected as indicators for characteristic soil properties since soil pH is considered a master variable in soils as it affects many chemical processes.

2.6 | Vegetation

The detrended correspondence analysis (DCA) of 31 relevés with in total 189 species was carried out using PC-ORD (Mc Cune & Mefford, 2011; Appendix S2). In the second matrix, we provided seven quantitative variables including the species number and the ordinal-scaled EIVs (Ellenberg et al., 2001) and designated the vegetation type from Walentowski et al. (2018) as a coding variable. EIVs use numerical values to express the average realized niches along six fundamental gradients (light availability [*L*], temperature [*T*], continentality [*C*], soil moisture [*M*], soil reaction [*R*], nutrients [*N*]). While the limitations and strengths of Ellenberg's approach have long been debated (e.g. Ewald, 2003), a number of studies showed good agreement between indicators and environmental variables (e.g. Schaffers & Sýkora, 2000; Schmidtlein & Ewald, 2003; Fanelli et al., 2007).

We calculated abundance-weighted EIVs for all species in a relevé (cf. Melman et al., 1988; Schaffers & Sýkora, 2000; Ellenberg, 2001). It is assumed that a species reaches a higher abundance when the environmental conditions at the site are nearer to the ecological optimum of a species (Käfer & Witte, 2004). The values were calculated after the exclusion of the planted coniferous species *Pinus sylvestris* and *Picea abies* and the tree layer to detect the differences in the undergrowth.

In this study we only present the Overlay Main Matrix graphing option (Peck, 2010, p. 120) for the responses of *M. sylvestris* and *M. domestica* × *sylvestris*.

2.7 | Gap fraction

The blue channel of hemispherical photos is considered to offer most contrast between sky and vegetation (Jonckheere et al., 2005), therefore it was extracted from the photos. The data was rescaled to 8-bit unsigned integer images. In a next step, an appropriate threshold value which divides sky pixels (brighter = higher intensity = higher values) from vegetation pixels (darker = less intensity = lower values), was determined. In a pre-analysis, the three algorithms "IJDefault" (Ridler & Calvard, 1978), "Otsu" (Otsu, 1979) and "Shanbhag" (Shanbhag, 1994) from the R package *autothresholdr* were found to perform best.

Which algorithm was eventually used was determined manually for each picture. Gap fraction was calculated as the fraction of white background (=sky) pixels within the picture. The four measurements of gap fraction at each tree site were analysed separately by cardinal direction, and also averaged by tree site (unweighted mean and median).

2.8 | Statistics

All data handling and statistical analysis were performed with the R software (R Core Team, 2018, version 4.0.3 Bunny-Wunnies Freak Out). A tree site mean value was calculated for pH, sand, silt and clay fraction, as well as the EIV.

The soil parameters were weighted by the depth of the corresponding horizon and the EIV by the cover percentage of each species in the shrub, herb or moss layer. The parameters were transformed to normality and homoscedasticity for statistical testing.

The (mean) EIVs of the considered vegetation layers and (mean) soil parameters as well as metadata and light measures were pairwise compared using Student's *t* tests and ANOVA (analysis of variance) on transformed dependent variables or the Mann–Whitney *U* test and post-hoc tests.

If a difference in distribution was visually identified from histograms, a two-sample Kolmogorov–Smirnov test was applied to test this. Furthermore, we tested the localities against each other due to locality-specific imbalance of soil and gap fraction parameters to avoid wrong significant signals using the Kruskal–Wallis-test.

2.9 | Logistic regressions

Finally, binomial logistic regressions were built in order to model the occurrence of *M. sylvestris* and the hybrid in the sampled environmental gradients. The model equation of a logistic regression is a logit transformation of a linear regression (Borcard et al., 2011).

The equation returns the probability to find a *M. sylvestris* s. str. individual at a given independent parameter value. For one independent variable the probabilities ideally result in a curve with a sigmoid shape from 0 (0% probability of finding a *M. sylvestris* s. str.) to 1 (100%) over the measured range of the independent variable.

Subsets of the environmental parameters (all indicator values, all soil parameters, all meta parameters, …) were used in several "full" models which were then subjected to a backwards selection. The parameters that appeared most promising in these models were combined to build an overall "full" model. *M. sylvestris* and *M. domestica* × *sylvestris* plots did not overlap, so a *M. sylvestris* plot was assumed to be an absence record for *M. domestica* × *sylvestris* and vice versa.

Accuracy of modelling, or prediction, was established via pseudo-r^2 (Nagelkerke, 1991; Hedderich & Sachs, 2016) and Akaike's Information Criterion (AIC; Akaike, 1974). Moreover, the receiver operating characteristic (ROC) was determined. This is a measure of classification accuracy based on repetitive thresholding. The area under a curve of true-positive vs false-positive classifications at all thresholds (AuROC) is calculated. If the AuROC value is about 0.5, the classification is random. Any value higher than 0.75 can be considered a fair classification (Fan et al., 2006).

3 | RESULTS

3.1 | Identification of *Malus sylvestris* and hybrids

The structure results of the investigated microsatellite patterns revealed that our samples belong to 22 *Malus sylvestris* and 11 *Malus domestica* × *sylvestris* specimens (Figure 2).

In locality BB there was an equal number of wild types and hybrids, whereas in the remaining localities investigated here, *Malus sylvestris* prevailed. To avoid locality bias in the interpretation of differences between *M. sylvestris* and *M. domestica* × *sylvestris*, influences of locality for soil and light have to be taken into consideration with additional tests (see below).

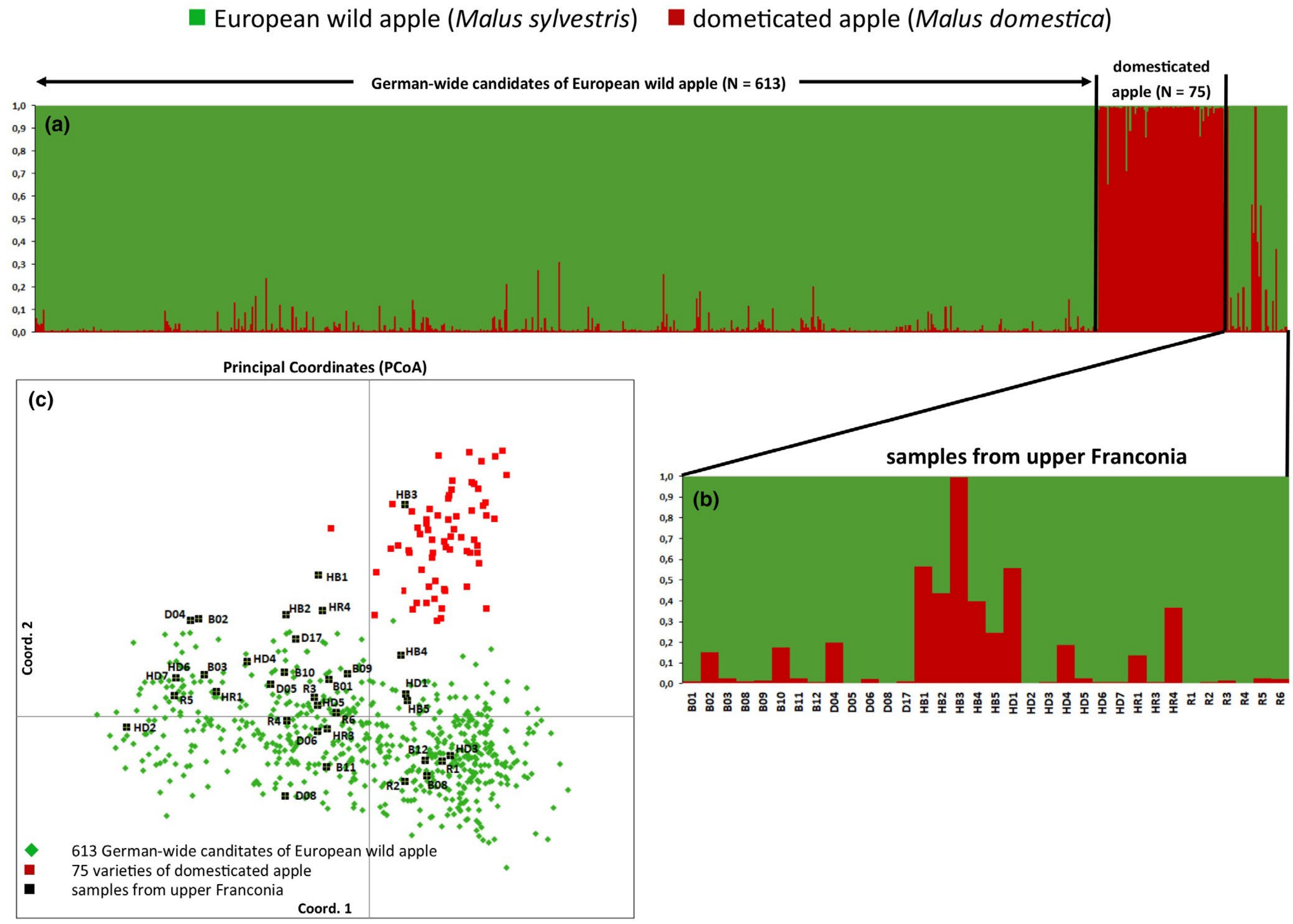


FIGURE 2 STRUCTURE results for the genetic affiliation of the investigated *Malus* samples using reference data consisting of 618 *M. sylvestris* individuals and 66 cultivars

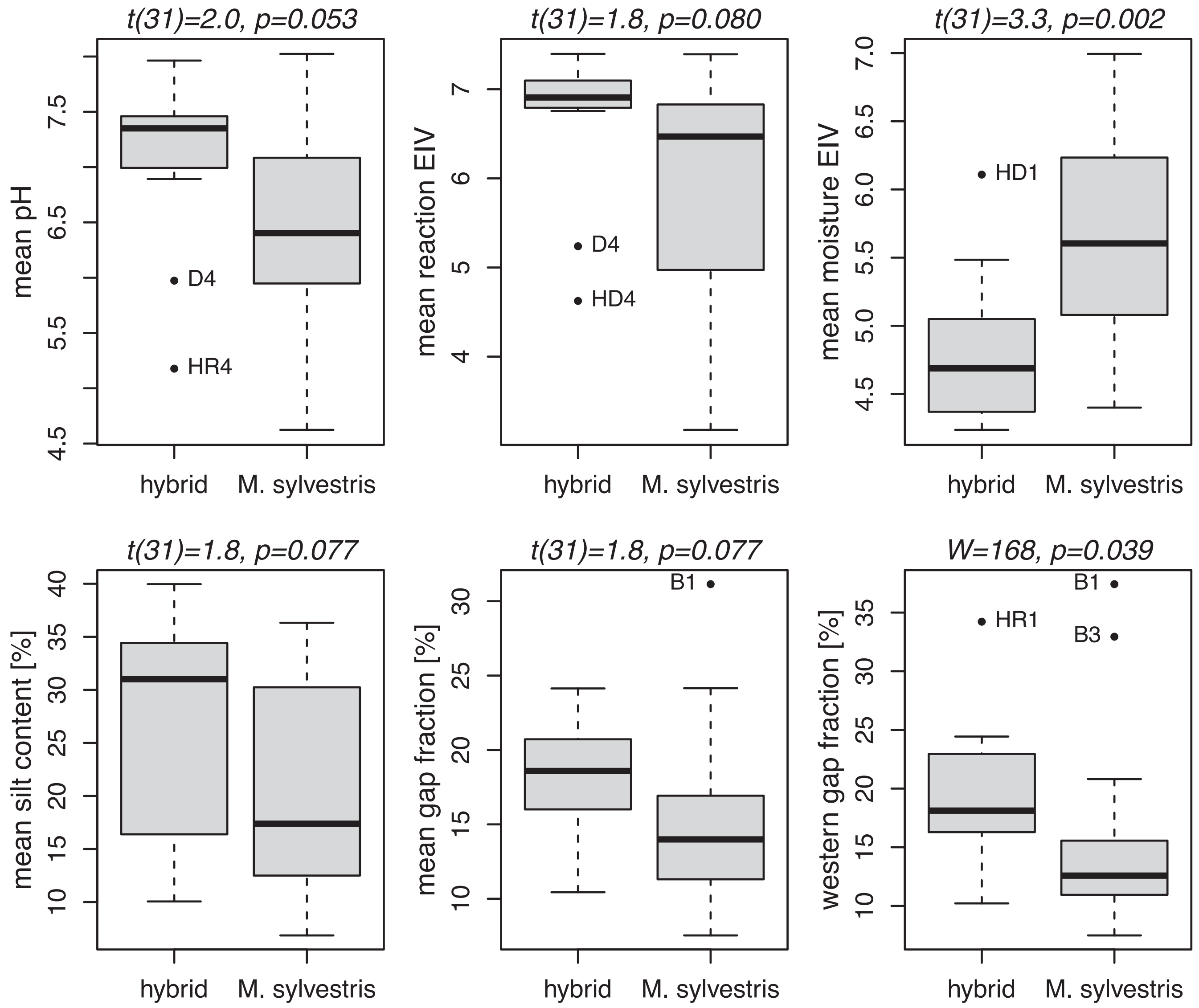


FIGURE 3 Values of parameters with significant differences between the stands of *Malus sylvestris* and its hybrid with *Malus domestica* for soil parameters and Ellenberg indicator values

3.2 | Locality differences for the investigated parameters

A Kruskal–Wallis test and an additional post-hoc test revealed significant differences ($p < 0.001$) of at least one locality (BB, DS, RMA) for the mean sand value (highest in RMA), the mean silt value (highest in BB) and in the mean pH value (highest in BB). For the mean clay value all localities were different, with locality DS having the highest, BB intermediate and RMA the lowest levels of clay in the soil. No significant differences were found in mean gap fractions between localities.

To analyse the ecologic significance of the investigated parameters both means and the frequency distribution were compared. Differences in parameter means of *M. sylvestris* and hybrid habitats could be found in soil and light parameters and in the mean EIVs *M* and *R* (Figure 3).

Figure 4 shows the frequency distribution of the parameters EIV *R*, mean gap fraction, mean silt and mean pH value for *M. sylvestris* and *M. domestica* × *sylvestris*. Although the means of silt fractions (Table 1) and pH value are significantly different, looking at the frequency distribution it becomes clear that a linear tendency of *M. sylvestris* to prefer one end of the parameter spectrum over the other can only be observed for the EIV *M*. Gap fractions are bimodal for *M. sylvestris*, meaning that it occurs in full overhead light but also in semi-shade conditions more often than the hybrid. Mean pH and mean silt content especially appear to be multimodally distributed for both taxa. From the frequency distributions of the soil parameters no clear preference of either the one or the other taxon can be deduced. Both taxa tolerate a wide range of pH values and silt levels. Only the high frequency of *Malus sylvestris* habitats at low pH values is remarkable, as it is known as a basophile species (Leuschner & Ellenberg, 2017). Comparison of both the means and

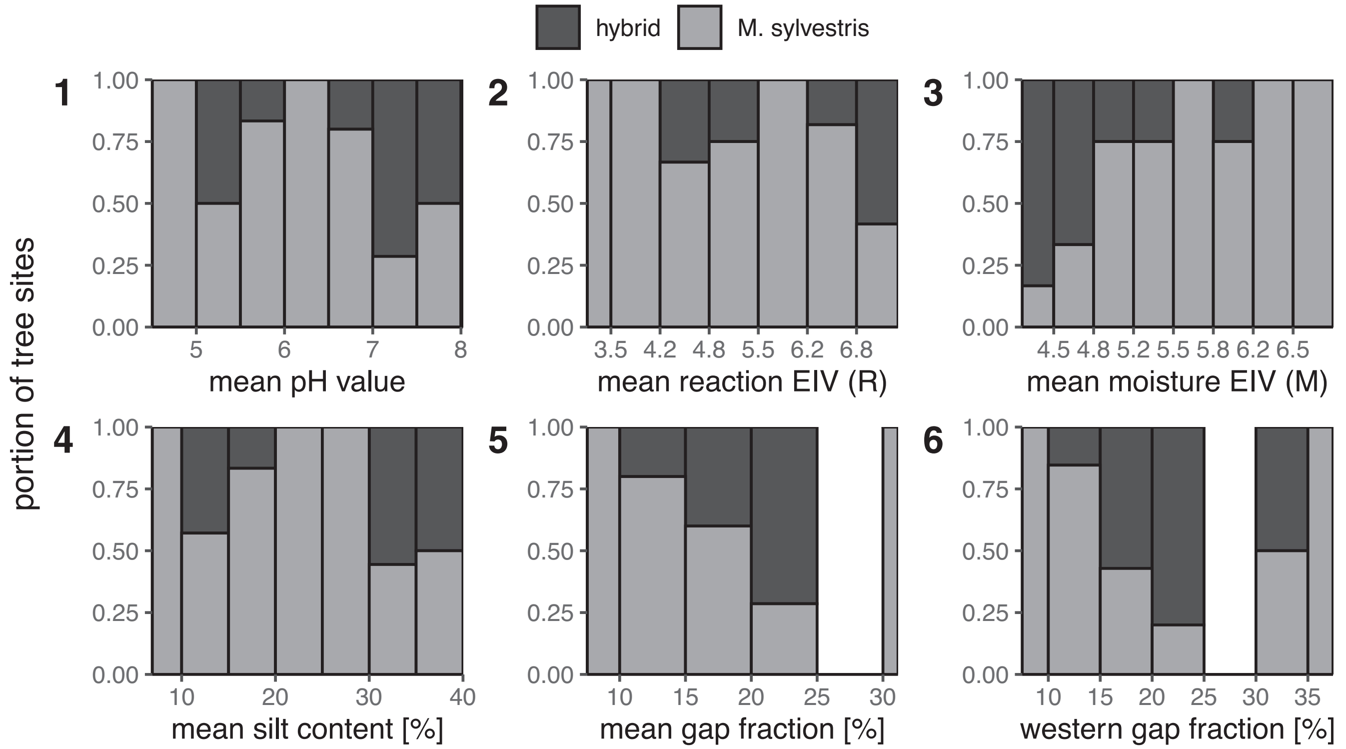


FIGURE 4 Frequency distribution of the parameters mean pH, mean reaction Ellenberg indicator value (EIV; *R*), mean moisture EIV (*M*), mean silt content, mean gap fraction, and Western gap fraction for *M. sylvestris* and *M. domestica* × *sylvestris*

frequency distribution show that *M. sylvestris* has a higher tolerance for growing in shadier conditions than *M. domestica* × *sylvestris*. The differences in the gap fraction data (mean and Western gap fraction, both significant at the 90% and 95% level respectively) are not influenced by the locality since locality-specific differences were not detected, meaning that the wild species is clearly more shade-tolerant than the hybrid. The EIVs are significantly different for moisture (*M*) and soil reaction (*R*). EIV *M* is not differentiated between the localities (Kruskal–Wallis not significant), i.e. all localities contribute to this differentiation, not only the floodplain locality RMA. The frequency distribution shows an equal and linear distribution. The higher moisture EIV is in line with the DCA data, indicating that *Malus sylvestris* is especially favoured on hydromorphic soils and wetland conditions (see below). While EIV *R* was only weakly correlated with measured pH (Schaffers & Sýkora, 2000), EIV *M* appeared to be a good indicator of average soil moisture contents in summer.

3.3 | Logistic regression

Several significant models were found. Accuracy was determined as mean correct classification in a cross-validation. The pseudo-r^2 value can be interpreted as a regular coefficient of determination though values >0.2 equal an acceptable model and >0.5 an excellent one (Hedderich & Sachs, 2016). The pseudo-r^2 values (Nagelkerke, 1991) ranged from 0.14 to 0.51. The accuracy in the cross-validation ranged from 52% to 78% and their area under the ROC curve (AuROC) was between 0.72 and 0.86. The closer the AuROC value is to 1, the better the classification. 0.5 would indicate randomness.

Models involving the mean moisture indicator proved the most promising (Table 2). Soil physico-chemical parameters explained very little of the observed deviance. Very high probabilities for *M. sylvestris* span the whole observed range of clay fractions, for example.

The best predictions for the distribution of *M. sylvestris* and *M. domestica* × *sylvestris* are offered by a binominal model using the EIV *M*. The second-best model used the combination of gap fraction West + EIV *M*. The model using the EIVs *C*, *M* and *N* also provided a good fit (Table 2).

In Figure 5 the modelled probabilities for *M. sylvestris* and *M. domestica* × *sylvestris* are plotted for EIV *M* and Western gap fraction, showing that under increasing soil moisture and decreasing light availability the probability for *M. sylvestris* increases and decreases for the hybrid.

3.4 | Plant communities

Detrended correspondence analysis was used to find the main factors or gradients that typify ecological community data (Figure 6) and was the basis for a joint Overlay Main Matrix of species abundances of *M. sylvestris* and *M. domestica* × *sylvestris* (Figure 6).

The DCA of the 31 wild apple plots with 189 species revealed five groups of relevés which were assigned to five communities as specified by Walentowski et al. (2018). Axis Eigenvalues are shown in Figure 6, and the linear regression of the fits and correlations for

TABLE 2 Model parameters of the best logistic regression models (independent parameters *xi*, slopes *ai* and intercept *b*) and predictive power assessments: Nagelkerke's pseudo-r^2, accuracy and area under the receiver operating characteristic curve (AuROC), as well as Akaike's Information Criterion (corrected for small samples [AICc])

Model	Intercept	Slope	Slope2	Slope3	Slope4	Nagelkerke's r^2	AuROC	Accuracy	AICc
EIV moisture	−9.548	1.978				0.379	0.831	0.779	35.887
Gap fraction West and EIV moisture	−7.992	−0.032	1.773			0.378	0.840	0.762	37.806
EIVs Continentality + moisture + nitrogen	2.387	−2.070	2.340	−1.020		0.479	0.864	0.721	37.471
M + pH	−7.763	1.878	−0.187			0.382	0.814	0.729	38.214

Abbreviations: EIV, Ellenberg indicator value; *M*, Ellenberg-soil moisture.

environmental variables are shown in Tables 3 and 4. The results suggest that the linear regression model provides an adequate fit to the data. Axis 1 explains 42% of the variation in floristic data, Axis 2 contributes to another 10% (cumulative >51%; Table 3).

The eigenvalue of 0.73 of Axis 1 (Figure 6) shows the strength of the floristic variability explained by environmental variables (Table 4).

The environmental variables *M* (soil moisture), *N* (nitrogen) and *R* (base saturation) were positively correlated with the first axis; *C* (continentality) was negatively correlated. Along the first axis, many plant communities surrounding hybrid and *M. sylvestris* trees respectively are also separated.

A joint Overlay Main Matrix of species abundances of *M. sylvestris* and *M. domestica* × *sylvestris* is presented in Figure 7. Indeed, the low values of Pearson's correlation coefficient *r* and Kendall's tau coefficient indicated weak (positive or negative) linear relationships with the ordination axes.

Nevertheless, the linear regression trend lines of *Malus sylvestris* and the hydrid tend to show opposing trends and suggest an increase of *Malus sylvestris* towards the wet edge of forests and a decrease of the hybrid with decreasing continentality. In addition, the envelope curve of *M. sylvestris* is slightly concave, lower at the mean (*m*), hence is bimodal. This implies that *M. sylvestris* tends to achieve its highest relative abundances towards the ends of the axes (towards extreme, marginal sites). In contrast, the envelope curve of *M. domestica* × *sylvestris* has a maximum at *m* and thus is unimodal with a right-skewed, positive distribution. Particularly striking were the divergent responses to eutrophic wet woodland habitats (*Stellario nemorum–Alnetum glutinosae*), where the hybrid was largely absent.

4 | DISCUSSION

In this study, we tried to shed light on the question whether *M. sylvestris* is threatened by replacement by the hybrid and find indications for differences in ecological niches. Delimitation of the hybrid against the wild apple is difficult, relaying only on morphology. Leaf traits such as glabrous leaf veins and surface on the leaf underside are not sufficient, hairiness of flowers also has to be taken into consideration, so alternance and season complicate determination. Therefore, genetic microsatellite data were used and compared to a Germany-wide dataset of *M. sylvestris* and cultivars to clearly identify the taxa. It became apparent that in the three investigated mixed populations the hybrid is much rarer than *M. sylvestris*, yielding 11 identified hybrid and 22 *M. sylvestris* specimens.

Comparison of the investigated ecological parameters showed that there are statistically significant differences between the habitats of *M. sylvestris* and the hybrid with *M. domestica*. Differences were found especially in light availability via mean and Western gap fractions, silt content of the soil, distribution of pH values as well as mean soil pH and in the EIVs soil humidity or moisture (*M*) and reaction (*R*). Our data support the hypothesis that habitat partitioning

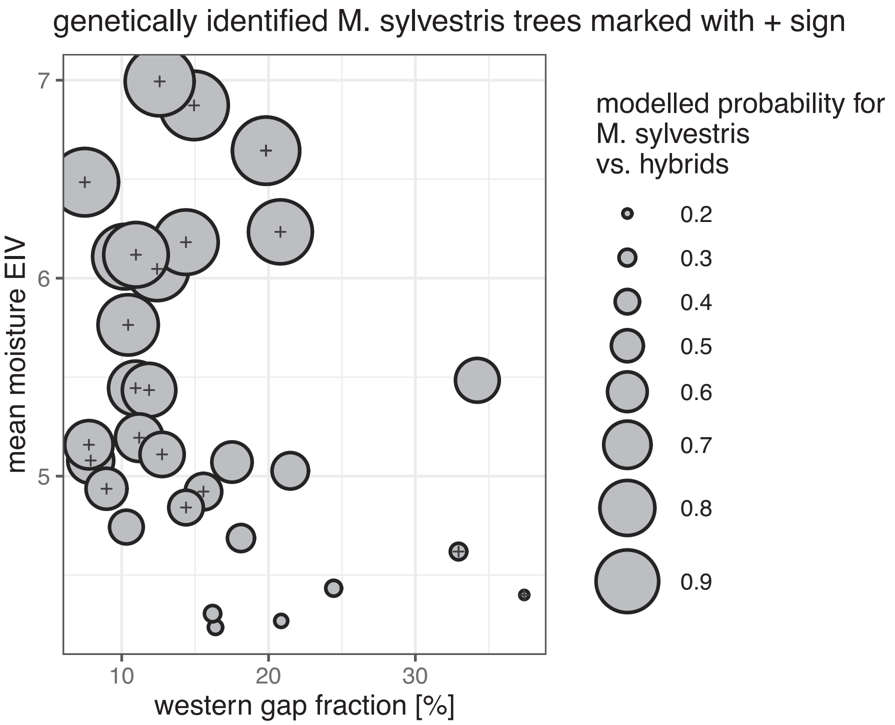


FIGURE 5 Model for *M. sylvestris* vs hybrid occurrence with mean Ellenberg indicator value (*T*) and eastern and southern gap fractions. European wild apple data sets are marked with a plus sign

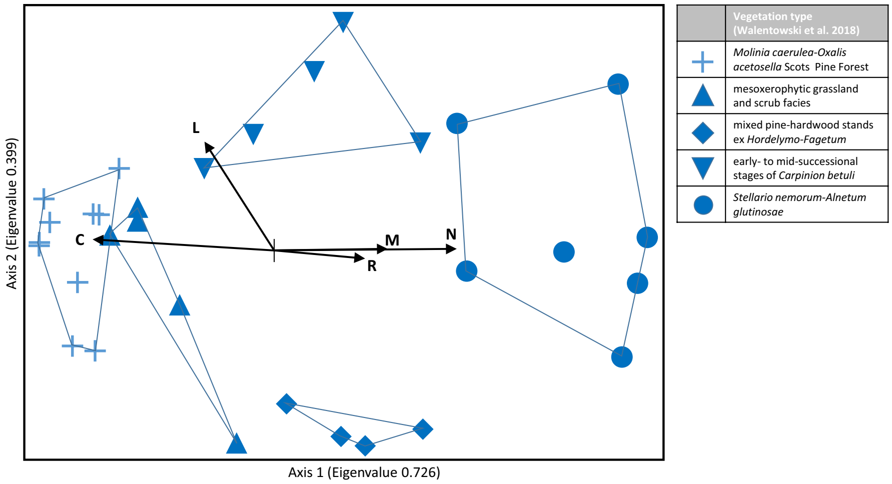


FIGURE 6 Detrended correspondence analysis (DCA) diagram of 31 wild apple plots recorded in 2017. Ordination of the plots is based on cover values of in total 189 species. Mean Ellenberg values for temperature (*T*), soil moisture (*M*), soil reaction (*R*) and nutrients (*N*) used as passive variables not affecting plot scores (cutoff r^2 value: 0.300); length of gradients axis 1: 4.43 *SD*, axis 2: 3.09 *SD*

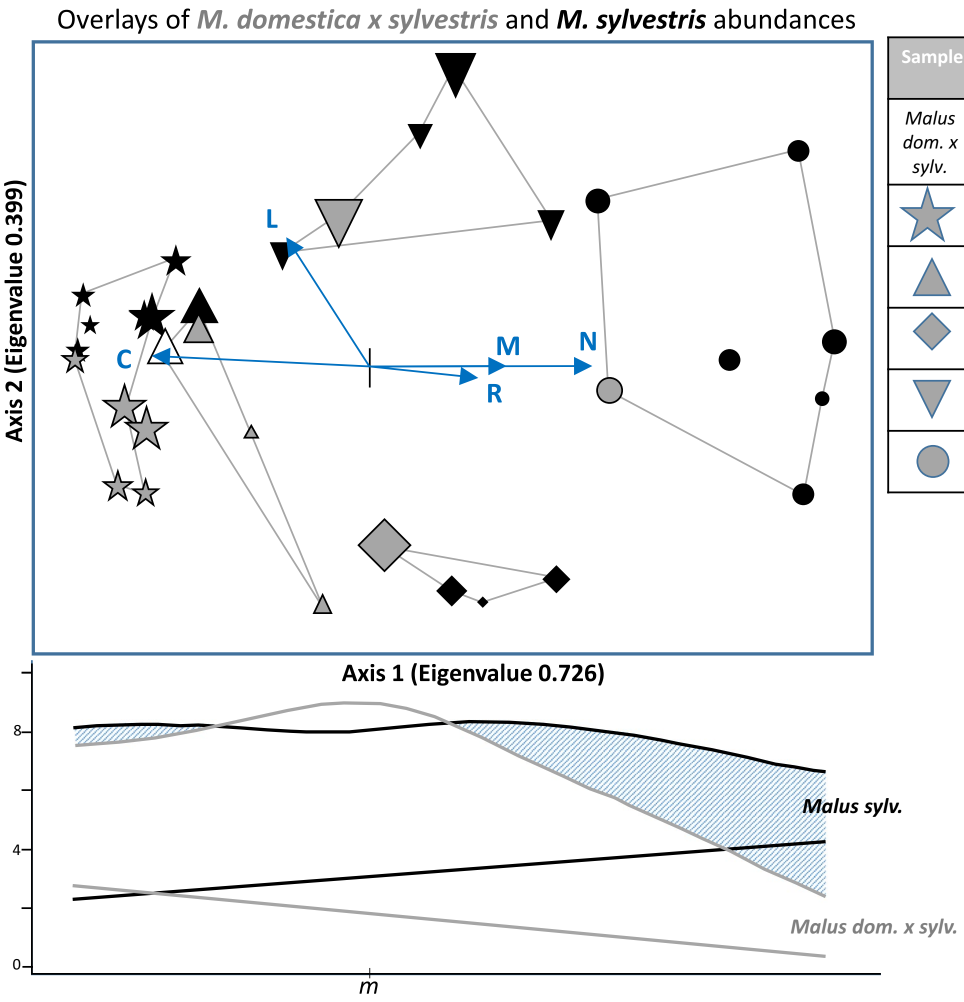
TABLE 3 Coefficient of determination (r^2) of the regression between distance in ordination space and Sørensen distance in the original floristic space (McCune & Grace, 2002)

Axis	r^2	Cumulative r^2
1	0.419	0.419
2	0.096	0.514
3	0.006	0.520

TABLE 4 Pearson correlation (*r*) of Ellenberg indicator values of the relevé samples with detrended correspondence analysis (DCA) axis 1 and 2

Axis	DCA axis 1	DCA axis 2
Ellenberg light avalailability [*L*]	−0.484	0.604
Ellenberg temperature [*T*]	−0.160	0.297
Ellenberg continentality [C]	−0.773	0.188
Ellenberg soil moisture [*M*]	0.621	0.056
Ellenberg soil reaction or pH [*R*]	0.551	−0.173
Ellenberg nutrients [*N*]	0.780	0.060

plays a substantial role as ecological constraint to interbreeding and is suitable for ensuring the long-term survival of the remaining allotopic populations of *M. sylvestris*. The central finding of the study was that *M. sylvestris* tolerates shadier and wetter conditions much better than the hybrid. Gap fractions of *M. sylvestris* were bimodally distributed, meaning that it copes with both shadier/lighter microhabitats than the hybrid (Figure 4). Especially shade tolerance is advantageous since it guarantees a longer survival of *M. sylvestris* under natural succession conditions when taller trees start to shade



Sample units of		Vegetation type (Walentowski et al. 2018)
Malus dom. x sylv.	*Malus sylv.*	
☆	★	*Molinia caerulea-Oxalis acetosella* Scots Pine Forest
△	▲	mesoxerophytic grassland and scrub facies
◇	◆	mixed pine-hardwood stands ex *Hordelymo-Fagetum*
▽	▼	early- to mid-successional stages of *Carpinion betuli*
○	●	*Stellario nemorum-Alnetum glutinosae*

Axis	*Malus dom. x sylv.*	***Malus sylv.***
1:	r= -,281 tau= -,211	r= ,259 tau= ,198
2:	r= -,224 tau = -,222	r= ,404 tau= ,288

FIGURE 7 Joint Overlay Main Matrix of species abundances of *M. sylvestris* and *M. domestica* × *sylvestris*. Scaled symbols for each sample unit is displayed proportionally to the relative abundance in each plot (large symbols indicate higher abundances). The bottom scatterplot shows the relationship between the Axis 1 ordination scores (on the *X* axis) and the abundance of the response (now on the *Y* axis) with a linear regression line drawn through the points (straight line) and 95% confidence envelope curves. The area shaded with a blue colour visualizes the remaining allotopic occurrences of *M. sylvestris* outside the niche overlappings towards the edges of the gradients

the understorey trees, which would first of all exclude the hybrid. This finding is in line with Janssen (2019), who recorded the wild apple more frequently in or at the edge of forests than in groves or hedges where in contrast the hybrid was found more frequently. There might be a trade-off between an advantageous sheltering effect and an adverse shading effect by the canopy for understorey trees. It is well known that fruit set of *M. sylvestris* might be favoured when diffuse light is sufficiently available (Binder et al., 2011); however, the authors point out that flower alternance leads to an overlay of those effects. The ability to form root suckers may even enhance the shade resistance advantage since *M. sylvestris* may thus survive periods of dense forest cover by renewing its ageing trunk and awaiting a reopening of the upper tree layers to fruit again.

The role of light in niche partitioning of *M. sylvestris* and *M. domestica* × *sylvestris* can be explained by its second parent, *M. domestica*. *M. domestica* was bred for orchard conditions where no canopy of larger trees dampens light supply and this high light demand may have been inherited to the hybrid. The tendency of *M. domestica* × *sylvestris* to grow on mesotrophic soil with higher silt fractions than typical at *M. sylvestris* sites may have also been inherited from the domesticated apple since its larger fruits compared to those of *M. sylvestris* would most likely require higher soil fertility.

According to the results of the DCA ordination (Figure 7), the statistical tests and the logistic regressions, the environmental filter was strongest for soil wetness. This accords well with recent research findings on *M. sylvestris* floodplain populations with low levels of admixture with *M. domestica*, compared to other habitats (Schnitzler et al., 2014; Wagner et al., 2014). Moreover, other studies found that forest habitats on shallow soils on calcareous rocks and screes exposed to the sun may have similar effects for maintenance of remaining allotopic populations of *M. sylvestris* by excluding the hybrid (Walentowski et al., 2018). Therefore, soil wetness and high nutrition supply are not required by *M. sylvestris*. Instead, the distribution of *M. sylvestris* is focussed to ecological maxima or minima, like water deficit/water surplus; nutrient poverty/nutrient surplus;

acid stress/lime excess (Schnitzler et al., 2014; Wagner et al., 2014; Walentowski et al., 2018). Our data highlight that *M. sylvestris* also tolerates much lower pH values than known before and can tolerate poor soils better than the hybrid. Conclusively, we have to highlight the importance of landscape heterogeneity for the conservation of genetic integrity of *M. sylvestris*.

A higher density of wild apples due to partial ecological exclusion of the hybrid with *M. domestica* also increases the probability for pollination by pure wild apples since most pollination takes place at small scales (see Reim et al., 2015). Thus, these effects, together with other post-zygotic barriers not known so far, may contribute to the conservation of non-admixed wild-apple populations. However, to reduce admixture the planting of cultivated apples (i.e. to compensate for ecosystem interference or nature conservation efforts) should be avoided in areas with existing *M. sylvestris* populations if not strictly needed for commercial or economic purposes. Moreover, the rare habitats of the wild apple with low levels of admixture such as intact floodplain forests and forest communities at dry and rocky soil should be strictly protected.

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AUTHOR CONTRIBUTIONS

MF, GA and LA conceived of the research idea. LA, aided by MF, collected the data. Microsatellite analyses and genetic characterization were carried out by AH. Statistical analysis was mainly conducted by LA with contributions from HW. LA, MF, HW and GA wrote the manuscript. All authors contributed in the discussion and provided comments.

DATA AVAILABILITY STATEMENT

Data are stored as electronical supplement of this publication.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

Appendix S1. Microsatellites of *Malus sylvestris* and *Malus domestica × sylvestris* for six microsatellite loci.

Appendix S2. Vegetation data with species occurrences in %.

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